National Aeronautics and Space Administration

Developmental Research in Space: Predicting Adult Neurobehavioral Phenotypes via Metabolomic Imaging

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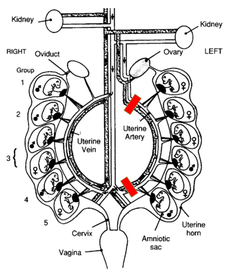
Introduction

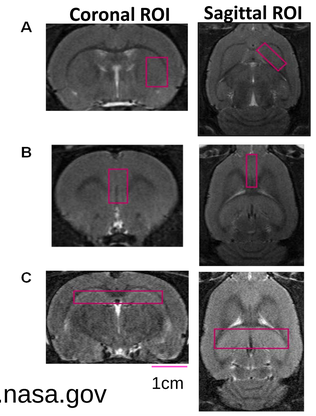
As interest in human habitation and eventual colonization of space increases, there is a need to understand mammalian reproduction and development beyond Earth. Our previous studies of pregnant rats suggest that, during vaginal birth, labor contractions may be weaker and less efficient following exposure to spaceflight as compared to ground controls[1] indicating a potential need for cesarean deliveries in the weightless space environment. Further, we predict that lung liquid clearance, a vital postpartum requirement for the rapid onset of pulmonary respiration at birth, will be significantly reduced in the space environment. On Earth, even moderate birth asphyxia is associated with adverse neurobehavioral outcomes. To advance methods for studying birth and development in space, we report here a new rodent model of cesarean delivery and moderate birth asphyxia. We utilized Magnetic Resonance Spectroscopic (MRS) imaging at one-week postnatal and behavioral assays at eight-weeks postnatal to test the hypothesis that neonatal neurometabolite profiles can predict adult anxiety profiles associated with birth asphyxia. Non-invasive MRS screening of neonatal offspring is likely to advance ground-based space analogue studies informing mammalian development in space, and achieving high-priority multigenerational research that will enable studies of the first truly 'space-developed' mammals.



Subjects were 22 SD rat offspring derived from 14 timed pregnant rat dams. On the day of expected birth (Gestational day [G]22), the uterus was externalized into a heated saline bath. Perinatal asphyxia (15min) was produced by occluding the blood supply feeding one of the dam's paired uterine horns for (red demarcations; Occluded; OCCL). The other uterine horn remained undisturbed (Non-Occluded; NOCCL). Pups from both horns were surgically delivered. Vaginally-born (VAG) offspring were used to control for birth mode. OCCL, N=8; NOCCL, N=8; VAG, N=6

Magnetic Resonance Spectroscopy

MR Images: 7T Magnet



Following exposure to perinatal asphyxia and/or C-section we measured postnatal neurometabolic profiles *in vivo* (^{1}H) MR Spectroscopy (MRS):

- ✧ **Energy Metabolism**: glutamine and glutamate (Glu+Gln)
- ✧ **Neuronal integrity**: n-acetylaspartate and n-acetylaspartate-aspartyglutamate (NAA+NAAG)
- ✧ **Myelination and acetylcholine turnover**: glycerophosphocholine and phosphocholine (GPC+PCh)
- ✧ **Synaptic plasticity**: Taurine (Tau)
- ✧ **Osmoregulator and glial marker:** Inositol (Ins)

Proton (^{1}H) magnetic resonance spectroscopy (left). MRS images were acquired from (A) Striatum, (B) Prefrontal Cortex, and (C) Hippocampus using a horizontal 7 T magnet interfaced with a digital spectrometer operating at a resonant frequency of 300 MHz (Bruker BioSpin, Billerica, MA). All proton MR spectra were acquired using previously published methods[3].

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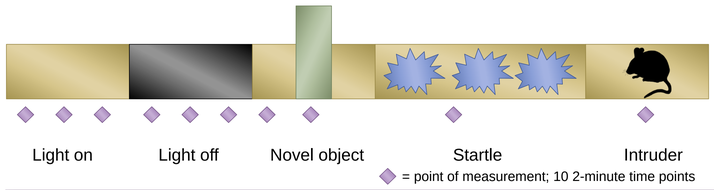
Behavioral Assay



Longitudinal Behavioral Analysis	Operational Definitions
Physical Activity	Ambulation, self-grooming, rearing, sniffing
Thigmotaxis	Time spent in outer border & center square
Defecation	Quantification of fecal droppings

Behavioral Tests	Operational Definitions
Novel Object	Approach, orientation, sniff, contact with plastic block
Startle Response	Freezing after auditory stimulus
Resident-Intruder Test	Sniffing, exploring and interacting with conspecific (same-sex non-litter mate intruder)

Longitudinal Behavioral Analysis

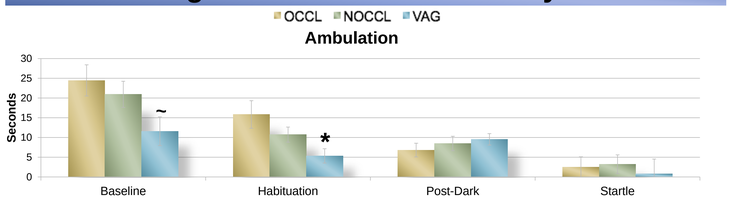


Figure 2. A trend in "Baseline" (p=0.076), and a birth mode effect in "Habituation" (p=0.046)

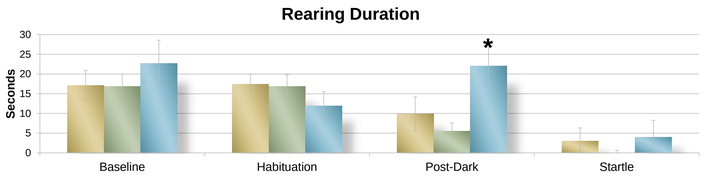


Figure 3. In "Post-Dark", we found a birth mode effect; NOCCL and VAG (p=0.021)

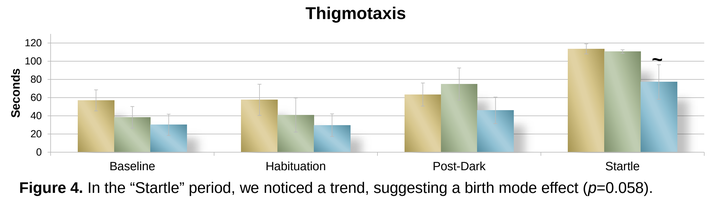


Figure 4. In the "Startle" period, we noticed a trend, suggesting a birth mode effect (p=0.058).

Novel Object Analysis

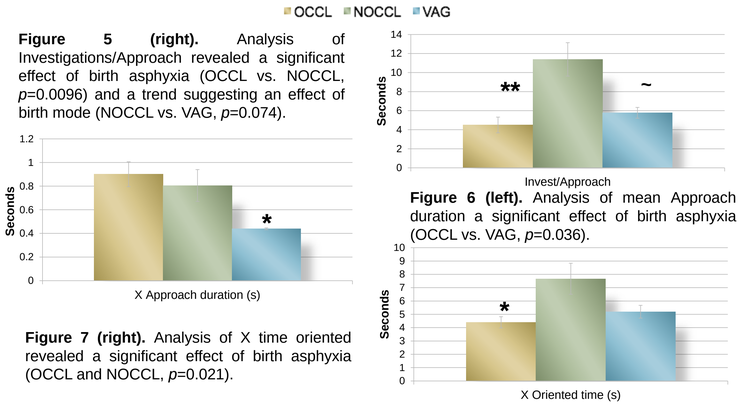


Figure 5 (right). Analysis of Investigations/Approach revealed a significant effect of birth asphyxia (OCCL vs. NOCCL, p=0.0096) and a trend suggesting an effect of birth mode (NOCCL vs. VAG, p=0.074).

Figure 6 (left). Analysis of mean Approach duration a significant effect of birth asphyxia (OCCL vs. VAG, p=0.036).

Figure 7 (right). Analysis of X time oriented revealed a significant effect of birth asphyxia (OCCL and NOCCL, p=0.021).

Neurometabolites Predict Novel Object Response

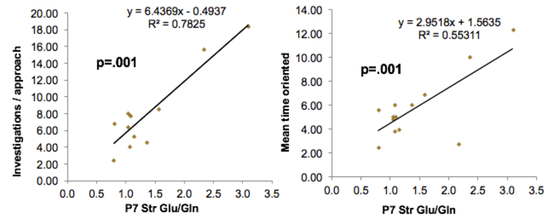


Figure 8. Investigations per novel object approach were significantly correlated with P7 striatal Glu/Gln (r^2=.78, p=.001). Mean time oriented was significantly correlated with Glu/Gln (r^2=.55, p=.001)[4].

Conclusions

These finding provide evidence that, following birth asphyxia, brain metabolite levels at one-week of postnatal age predict anxiety profiles in later life. Comparisons of offspring delivered by surgical cesarean section and vaginally delivered offspring (OCCL and NOCCL vs. VAG) revealed a secondary effect of birth mode, suggesting a potential influence of maternal anesthesia and surgical manipulation during the perinatal period on later life outcomes. These findings provide new insights into, and establish experimental procedures applicable to, studies of mammalian birth and development in space.

References & Acknowledgements

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I would like to thank my mentor Dr. April Ronca of the Reproduction lab for her guidance, and NASA and OSSI for making this possible. This research was supported by HD050201, NASA NNA04CK83, NASA Space Biology Program, and the Wake Forest Medical School Center for Biomolecular Imaging.